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Genomic characterization and evolutionary analysis of Chikungunya virus strains in Guangzhou, China, 2025

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Abstract

Background Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that has caused multiple widespread outbreaks and poses a significant public health threat. Recently, CHIKV outbreaks were reported in Guangdong Province, China. However, genomic data and information on local transmission remain limited.

Methods We collected 19 CHIKV-positive samples from Guangzhou and carried out whole-genome sequencing via the Oxford Nanopore platform. The obtained sequences were subjected to mutational and phylogenetic analysis. Furthermore, amino acid substitutions during transmission were analyzed via DynaMut2 to assess their potential effects on protein stability.

Results Phylogenetic analysis revealed that all 19 isolates belonged to the East/Central/South African (ECSA) lineage and were closely related to the S27b03 strain (GenBank accession: PV685524.1) from Réunion Island. Compared with S27b03, all the isolates carried a c.3879 C > T substitution. Following subsequent transmission, an additional c.7434G > A substitution appeared in ten isolates. Several nonsynonymous mutations were detected, including p.G230R, p.R491Q, and p.Q528L in nsP1; p.V587M in nsP2; p.A180T in nsP4; p. I54V in E2; p.A47V in 6 K; and p.V220A in E1. Structural prediction via DynaMut2 revealed that nsP1-G230R, nsP4-A180T, 6 K-A47V, E1-V220A, and E2-I54V decreased protein stability (ΔΔG ≤ -0.5 kcal/mol).

Conclusions Our results indicate that CHIKV isolates from Guangzhou belong to the ECSA lineage and are genetically close to the S27b03 strain. During local transmission, gene mutations and amino acid substitutions are observed. These findings provide useful information for understanding CHIKV spread and may help improve surveillance and control efforts.

Keywords Chikungunya virus, Genomics, Phylogenetic analysis, Local transmission

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Introduction

Chikungunya virus (CHIKV) is an arbovirus with a single-stranded, positive-sense RNA genome that is spread by mainly *A. aegypti* and *A. albopictus* mosquitoes [1]. Infection can lead to chikungunya fever, which typically presents with sudden fever, severe joint pain, and muscle aches [2, 3]. Although some studies have investigated treatments for CHIKV [4, 5], no specific antiviral drugs are currently approved for clinical use.

The CHIKV genome is approximately 12,000 nucleotides long and encodes four nonstructural proteins (nsP1-nsP4) and five structural proteins (C-E3-E2-6 K-E1). Nonstructural polyproteins play key roles in viral replication and host adaptation, whereas structural polyproteins are essential for receptor recognition, membrane fusion, and virion assembly [6, 7]. The envelope glycoproteins E1 and E2 form heterodimers that mediate target cell infection [8]. E1 mediates membrane fusion in acidic pH conditions within the endosome. Acidification triggers the dissociation of the E2-E1 complex, followed by E1-mediated fusion and release of the viral genome into the cytoplasm [9, 10]. E2 helps the virus attach to host cells by binding to receptors, as supported by structural studies [11]. Several mutations are known to increase CHIKV infectivity and transmission. For example, the E1-A226V mutation increases viral fitness in *A. albopictus* [12]. Similarly, the E2-L210Q and E2-I211T substitutions have been shown to improve dissemination efficiency in this mosquito species [13, 14]. Among the nonstructural proteins, nsP1 mediates membrane association and facilitates the capping of viral RNA [15]; nsP2 exhibits protease and helicase activities and recognizes the subgenomic RNA promoter [16, 17]; nsP3 interacts with translational regulators and facilitates the formation of replication complexes [18]; and nsP4 functions as an RNA-dependent RNA polymerase [19, 20]. Together, these proteins control viral replication and spread.

CHIKV was first identified in Tanzania in 1952 and has since caused many outbreaks worldwide. Currently, CHIKV is classified into three major genotypes: East/Central/South African (ECSA), West African (WA), and Asian strains [21]. In 2004, an outbreak caused by the ECSA genotype originated in Kenya and subsequently spread to Réunion Island, where it further evolved into the ECSA-derived Indian Ocean Lineage (IOL) [22–25]. This lineage has undergone important changes, including the E1-A226V mutation, which makes it easier for the virus to spread through *A. albopictus* mosquitoes [12]. Facilitated by this adaptation, the IOL strain rapidly spread throughout the Indian Ocean region, South Asia, Southeast Asia, and even parts of Europe [26].

In recent years, chikungunya fever has continued to spread globally, with outbreaks reported in multiple regions. Data from the European Centre for Disease Prevention and Control indicate that from early 2025 to July, approximately 240,000 cases of chikungunya virus infection were recorded across 16 countries and territories [27]. In China, CHIKV has been reported in several provinces. For example, imported and local cases were detected in Guangdong in 2010, and in Zhejiang in 2017 and 2019 [28–30]. The subtropical climate of Guangzhou enables sustained *Aedes* mosquito activity, and extensive domestic and international travel further heightens its vulnerability to arboviral outbreaks. Currently, an ongoing outbreak is occurring in Guangzhou city. However, the genetic characteristics and evolutionary background of the circulating strains remain unclear.

In this study, we performed reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to detect CHIKV infection in samples and identified 19 positive cases in Guangzhou. Whole-genome sequencing and phylogenetic analysis revealed that these strains belong to the ECSA lineage and are closely related to the S27b03 strain from Réunion Island. Comparative genomic analysis identified multiple nucleotide substitutions, among which the c.3879 C > T change was consistently present in all the isolates. Furthermore, several nonsynonymous mutations were identified, including G230R in nsP1, A180T in nsP4, A47V in 6 K, V220A in E1, and I54V in E2. These mutations may influence protein stability and conformational dynamics.

Materials and methods

Sample collection

Between 18 July and 13 August 2025, 19 CHIKV-positive serum samples were collected in Guangzhou for genomic analysis. The majority of samples were obtained from Huangpu District, with the remaining samples from Zengcheng, Tianhe, and Yuexiu districts. Detailed information for each sample is provided in Table 1.

RT-qPCR

The extraction of viral RNA from the CHIKV isolates was performed using the kit (DAAN GENE, Cat. #DA0620) according to recommended procedures. RT-qPCR was carried out with a DAAN GENE kit (Cat. #DK0110M) with the following primers and probes: CHIKV-FP (5'-CCGGCGACCATTTGYGATC-3'), CHIKV-RP (5'- C TCTGGTTCAGCCCCACC-3') and CHIKV-P (5'-CCG GAGGATGCACAGAAGCTGTT-3'). Each 25 μL reaction contained 20 μL of RT-qPCR buffer and 5 μL of RNA template. Amplification was performed on an Applied Biosystems 7500 system under the following cycling conditions: reverse transcription at 50 °C for 8 min, initial denaturation and enzyme activation at 95 °C for 2 min, followed by 40 cycles of 94 °C for 2 s and 58 °C for 29 s. Data were collected and analyzed using Applied Biosystems software.

Table 1 Summary of CHIKV samples included in this study

Isolate_ID	Location (District)	Collection date	Ct value
HP_001	Huangpu	2025-07-22	24.2
HP_002	Yuexiu	2025-08-06	29.1
HP_003	Huangpu	2025-08-12	20.3
HP_004	Huangpu	2025-08-13	20.2
HP_005	Zengcheng	2025-08-13	21.3
HP_006	Huangpu	2025-07-18	20.3
HP_007	Tianhe	2025-08-11	24.8
HP_008	Huangpu	2025-07-30	22.0
HP_009	Huangpu	2025-08-04	19.0
HP_010	Zengcheng	2025-08-13	22.9
HP_011	Huangpu	2025-08-05	22.8
HP_012	Huangpu	2025-08-09	20.7
HP_013	Huangpu	2025-08-12	26.4
HP_014	Yuexiu	2025-08-12	27.5
HP_015	Huangpu	2025-08-01	24.0
HP_016	Huangpu	2025-07-30	23.4
HP_017	Tianhe	2025-08-08	23.9
HP_018	Huangpu	2025-08-08	20.9
HP_019	Huangpu	2025-08-07	24.2

Nanopore sequencing

Library preparation was performed according to the manufacturer's instructions using the CHIKV amplicon library preparation kit (Cyanines Biotechnology, Guangzhou, China; Cat# RBK-CHIKV-001). Briefly, total RNA was reverse transcribed into cDNA using the RT mix. The cDNA was subsequently amplified by PCR using specific primers (Pool 1/2) to generate target fragments. Amplification was performed under the following cycling conditions: initial denaturation at 98 °C for 30 s, followed by 35 cycles of 98 °C for 15 s, 61 °C for 2 min, and 65 °C for 2 min, with a final extension at 65 °C for 2 min. PCR products were purified using magnetic beads. Barcoding was performed using the Rapid Barcoding Kit 24 (Oxford Nanopore Technologies, SQK-RBK114.24) according to the ONT protocol, after which sequencing adapters were ligated. The prepared libraries were loaded onto a flow cell (ONT, R10.4.1) and sequenced on a GridION platform. Each sequencing run lasted approximately 2 h, and each sample generated on average > 10 Mb of raw Nanopore data. Raw sequencing data were basecalled using Dorado (v0.6.2) with the Super-Accurate (SUP) model dna_r10.4.1_e8.2 400bps_sup@v4.3.0. The initial quality control of the raw FASTQ files was conducted using NanoPlot (v1.46.1) [31]. After filtering with a Q12 threshold, reads were aligned to the Chikungunya virus reference genome (strain S27b03, GenBank accession: PV685524.1) using minimap2 (v2.18-r1015) [32]. The resulting SAM files were converted into coordinate-sorted BAM files using SAMtools (v1.18) [33]. Variants were called using BCFtools (v1.17) [34]. Finally, a high-quality consensus sequence was generated using BCFtools consensus. Per-sample sequencing metrics are provided in Additional file 1.

Sequence analysis

Alignments of nucleotide sequences against selected alphavirus sequences were performed using MAFFT v7.525 with default parameters (gap opening penalty = 1.53, offset value = 0.123) [35]. Synonymous and nonsynonymous substitutions were identified with SnapGene 4.3.6 software. Phylogenetic analysis was conducted by constructing a maximum likelihood (ML) tree using MEGA 11 software under the General Time Reversible (GTR) nucleotide substitution model with a proportion of invariant sites (I) and gamma-distributed rate variation among sites (G4) [36]. Branch support was assessed using 1000 bootstrap replications. A total of 55 representative CHIKV reference sequences covering all major genotypes were included in the phylogenetic analysis together with the 19 newly sequenced strains generated in this study [37]. Detailed information of the reference sequences, including accession numbers, collection countries, years, and genotypes, is provided in Additional file 2. The complete multiple sequence alignment in FASTA format used for tree construction is provided as Additional file 3.

Prediction of protein stability

The effect of nonsynonymous CHIKV mutations on protein stability was evaluated using DynaMut2 (https://biosig.lab.uq.edu.au/dynamut2/) [38]. The output was expressed as ΔΔG_Stability (kcal/mol), where a negative ΔΔG value indicates that the mutation decreases protein stability. Input structures were generated with AlphaFold3 (https://alphafoldserver.com/) [39].

Results

Genomic and phylogenetic analysis

Recently, CHIKV has shown rapid local spread in Guangzhou. We identified 19 positive cases with Ct values < 30. The 19 CHIKV-positive samples, collected in Guangzhou between 18 July and 13 August 2025, were from Huangpu, Zengcheng, Tianhe, and Yuexiu districts and are summarized in Table 1. To characterize the viral genomes, we extracted viral RNA and performed whole-genome sequencing of all 19 CHIKV isolates via Oxford Nanopore Technology. Across all samples, the mean read depth was 730×. Each sample had a read depth greater than 100× and achieved 100% genome coverage at > 20× depth. Detailed sequencing metrics are summarized in Additional file 1. The assembled genomes encoded the expected nonstructural polyprotein (7,425 nt; nsP1-nsP4) and structural polyprotein (3,747 nt; C-E3-E2-6 K-E1). Notably, all the viral isolates carried the E1-A226V and E2-L210Q/I211T mutations, which have previously been

associated with enhanced transmissibility and persistence in *A. albopictus* mosquitoes [12–14].

To investigate the evolutionary relationships of the 19 CHIKV isolates from Guangzhou, a maximum-likelihood phylogenetic tree was constructed that included representative strains from the major lineages reported in previous studies [37]. Phylogenetic analysis showed that all 19 CHIKV isolates belonged to the ECSA genotype, a lineage associated with recent outbreaks in Asia, Africa, and the Indian Ocean region [26]. Notably, topological analysis revealed that all the Guangzhou isolates formed a clade most closely related to the S27b03 strain from Réunion Island, France (Fig. 1) [40]. This relationship suggests that the Guangzhou isolates may share an epidemiological origin with viruses circulating in the Indian Ocean region.

Gene mutation analysis in CHIKV isolates

To better understand the molecular features and transmission patterns of the 19 CHIKV isolates from Guangzhou, we performed a comparative mutational analysis using PV685524.1 as the reference sequence (Table 2). All 19 isolates carried a conserved synonymous substitution C > T at genomic position 3879, indicating that these viruses may share a common origin. This mutation was present at 100% frequency in all isolates, with a mean coverage of 726× at this position. Further analysis identified an additional G > A nucleotide substitution at position 7434 in ten isolates (HP_003, HP_005, HP_004, HP_010, HP_009, HP_019, HP_011, HP_018, HP_001, HP_015), suggesting the emergence of a sub-lineage during local spread. This c.7434G > A substitution lies within the nsP4-C junction and does not alter the viral protein sequence.

Phylogenetic analysis was consistent with these findings, showing that all the isolates grouped into a single clade, with those carrying the 7434G > A variant forming a distinct subcluster (Fig. 2). Notably, isolate HP_019 exhibited an A > T mutation at position 1583, which was absent in HP_003, HP_005, and HP_009, suggesting a transmission link among these cases. These results indicate that the outbreak likely originated from a single introduction, followed by the progressive accumulation of mutations during subsequent community transmission.

Amino acid substitutions

The amino acid sequence is fundamental to protein structure and function. To evaluate the potential impact of the nucleotide changes identified in the 19 CHIKV isolates, we analyzed their amino acid variations using PV685524.1 as the reference (Table 2). Both the C3879T and G7434A substitutions were predicted not to affect the encoded proteins, as C3879T is synonymous and G7434A occurs in a noncoding region. In the E1 protein, valine at position 226 (E1-A226V) was conserved in all the isolates, a mutation linked to increased CHIKV transmission in *A. albopictus* [12]. A novel substitution, p.V220A was identified in the E1 protein of isolate HP_002. Additional amino acid mutations were detected in several isolates. These included p.G230R in nsP1 of HP_001, which has been linked to resistance against the antiviral compound 6-fluoro-homoneplanocin A (FHNA) [41]. Other substitutions included nsP2 p.V587M in HP_016, nsP1 p.R491Q in HP_008, nsP1 p.Q528L and nsP4 p.A180T in HP_019, 6 K p.A47V in HP_012, E2 p.I54V in HP_014, and nsP4 p.A180T in HP_009.

To assess the potential impact of amino acid substitutions on the stability and function of CHIKV proteins, DynaMut2 was used to predict changes in the Gibbs Free Energy (ΔΔG, kcal/mol). The predicted values are summarized in Table 3. Overall, some variants were estimated to have modest effects on protein stability. In contrast, nsP1 p.G230R, nsP4 p.A180T, 6 K p.A47V, E2 p.I54V, and E1 p.V220A presented $|\Delta\Delta G| \geqq 0.5$ kcal/mol, suggesting potential perturbations in local stability or conformational dynamics. To further illustrate these findings, the local interaction networks of these variants were visualized (Fig. 3), providing structural support for their potential influence on stability and conformational dynamics.

Discussion

Currently, CHIKV is spreading in urban areas of Guangdong Province, China [42]. However, the genomic information and molecular epidemiological features of CHIKV isolates from Guangzhou remain poorly characterized. In this study, we performed a detailed genomic analysis of the circulating CHIKV strains to clarify their genetic characteristics and evolutionary patterns. These results provide preliminary molecular epidemiological insights into the circulating CHIKV strains in Guangzhou. In addition, the phylogenetic analysis and observed mutation patterns provide preliminary information that could inform future investigations into the potential origin of the CHIKV strains prevalent in Guangdong Province.

In this study, all 19 CHIKV isolates were classified within the ECSA lineage and were closely related to the S27b03 strain originating from Réunion Island. Réunion Island experienced a large outbreak of CHIKV in 2005–2006, after which only sporadic cases were reported until viral activity was detected again in August 2024 [40, 43, 44]. With increasing global travel and trade, strains originating from Réunion Island have spread globally. For example, a 2025 report documented seven imported cases in Europe among travelers from Réunion Island, with sequencing confirming that these viruses belong

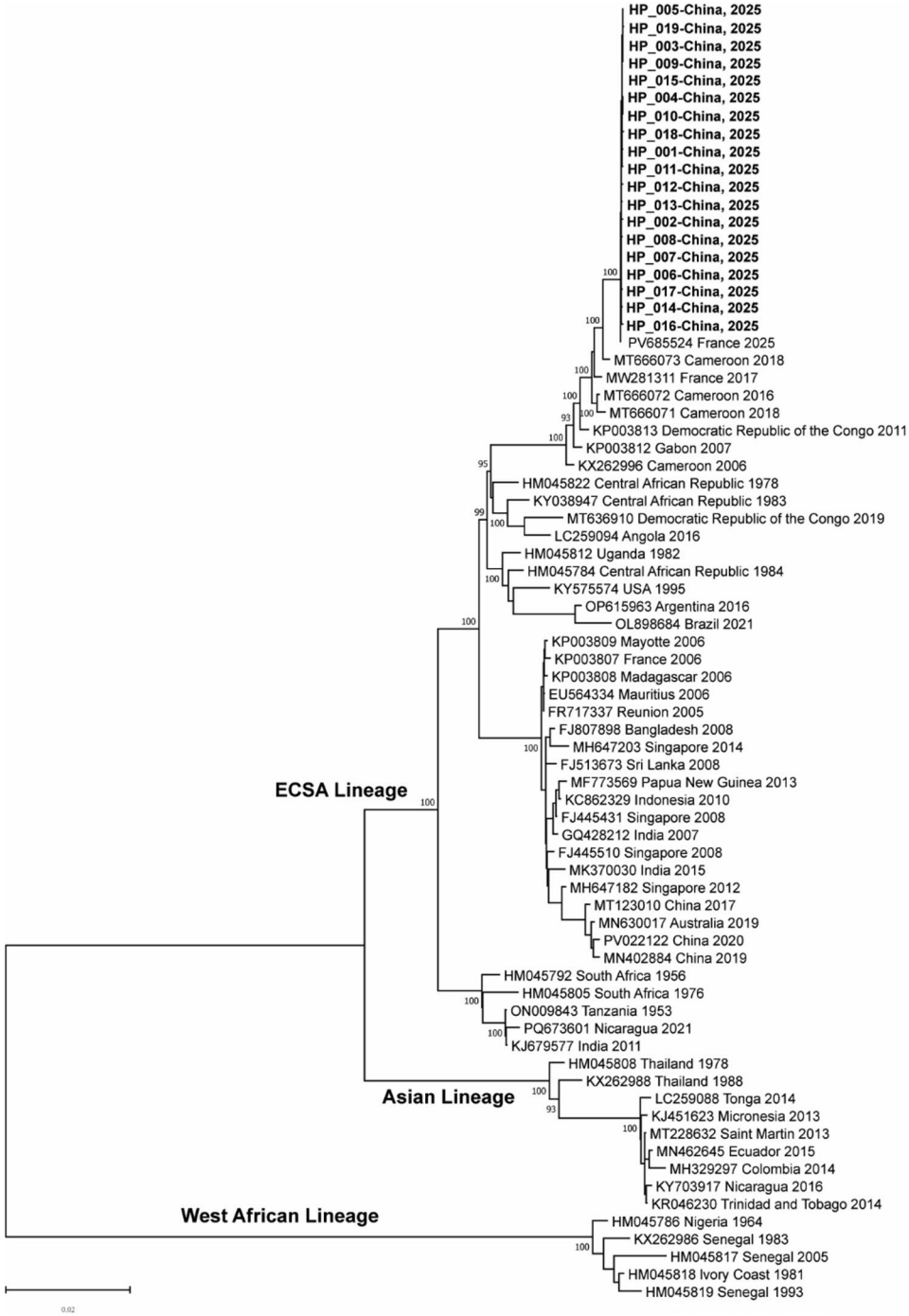

Fig. 1 Phylogeographic tree of representative full-length CHIKV genomes using Maximum-Likelihood method. Viruses are represented by GenBank accession numbers, country names in which the samples were obtained, and collection years. The viruses that explored in this work are in tips and colored accordingly. Bootstrap values are shown for key nodes

to the ECSA lineage [37]. Thus, analyzing the genetic and evolutionary characteristics of local strains helps us understand how the virus spreads and is distributed.

All 19 local CHIKV genomes obtained from Guangzhou carried the c.3879 C > T substitution, suggesting a likely single introduction of the virus into the region. An additional c.7434G > A change subsequently appeared, giving rise to a distinct monophyletic subclade that included isolates HP_003, HP_005, HP_004, HP_010, HP_009, HP_019, HP_011, HP_018, HP_001, and HP_015. This stepwise accumulation of mutations mirrors patterns documented in earlier outbreaks.

Table 2 CHIKV nucleotide and amino acid mutation profiles

Isolate_ID	Nucleotide_Change	Regions	Protein Change	Read depth at variant position (×)
HP_001	c.688G > A	nsP1	G230R	258
	c.3879 C > T	nsP2	Syn.	127
	c.7434G > A	nsP4-C junction	--	124
HP_002	c.1411T > C	nsP1	Syn.	752
	c.3879 C > T	nsP2	Syn.	899
	c.10576T > C	E1	V220A	939
HP_003	c.3879 C > T	nsP2	Syn.	737
	c.6127G > A	nsP4	A180T	523
	c.7434G > A	nsP4-C junction	--	581
HP_004	c.3879 C > T	nsP2	Syn.	211
	c.7434G > A	nsP4-C junction	--	173
	c.9416 C > T	E2	Syn.	330
HP_005	c.3879 C > T	nsP2	Syn.	385
	c.6127G > A	nsP4	A180T	298
	c.7434G > A	nsP4-C junction	--	287
HP_006	c.3879 C > T	nsP2	Syn.	1761
HP_007	c.3879 C > T	nsP2	Syn.	529
HP_008	c.1472G > A	nsP1	R491Q	161
	c.3879 C > T	nsP2	Syn.	119
HP_009	c.3879 C > T	nsP2	Syn.	1668
	c.6127G > A	nsP4	A180T	993
	c.7434G > A	nsP4-C junction	--	721
HP_010	c.3879 C > T	nsP2	Syn.	213
	c.7434G > A	nsP4-C junction	--	122
	c.9416 C > T	E2	Syn.	239
HP_011	c.690G > A	nsP1	Syn.	1537
	c.3879 C > T	nsP2	Syn.	833
	c.7434G > A	nsP4-C junction	--	361
HP_012	c.3879 C > T	nsP2	Syn.	394
	c.4308T > C	nsP3	Syn.	438
	c.9874 C > T	6 K	A47V	357
HP_013	c.3879 C > T	nsP2	Syn.	329
	c.9362T > C	E2	Syn.	379
HP_014	c.3879 C > T	nsP2	Syn.	132
	c.8625 A > G	E2	I54V	105
HP_015	c.3879 C > T	nsP2	Syn.	493
	c.7434G > A	nsP4-C junction	--	418
HP_016	c.1173 C > T	nsP1	Syn.	297
	c.3364G > A	nsP2	V587M	132
	c.3879 C > T	nsP2	Syn.	109
HP_017	c.3879 C > T	nsP2	Syn.	1003
	c.9035 C > T	E2	Syn.	1132
HP_018	c.3879 C > T	nsP2	Syn.	2805
	c.4227T > C	nsP3	Syn.	3074
	c.7434G > A	nsP4-C junction	--	1085
	c.8837 A > G	E2	Syn.	887
HP_019	c.1583 A > T	nsP1	Q528L	1336
	c.3879 C > T	nsP2	Syn.	1053
	c.6127G > A	nsP4	A180T	769
	c.7434G > A	nsP4-C junction	--	395

Schuffenecker et al. documented stepwise evolution during the Indian Ocean outbreak. The reconstruction of the ancestral genotype of Réunion Island indicated that the founder virus diversified through four successive stages, eventually yielding three consensus sequences characterized by 14 polymorphic sites [45]. Our results provide useful insights into the possible origin of the virus and its circulation patterns during the sampling period. However, the samples in this study were collected over a relatively short period (18 July to 13 August 2025), which limits the temporal resolution of our analysis. Future studies with a longer sampling period and a larger sample size will be important to more comprehensively assess CHIKV transmission dynamics.

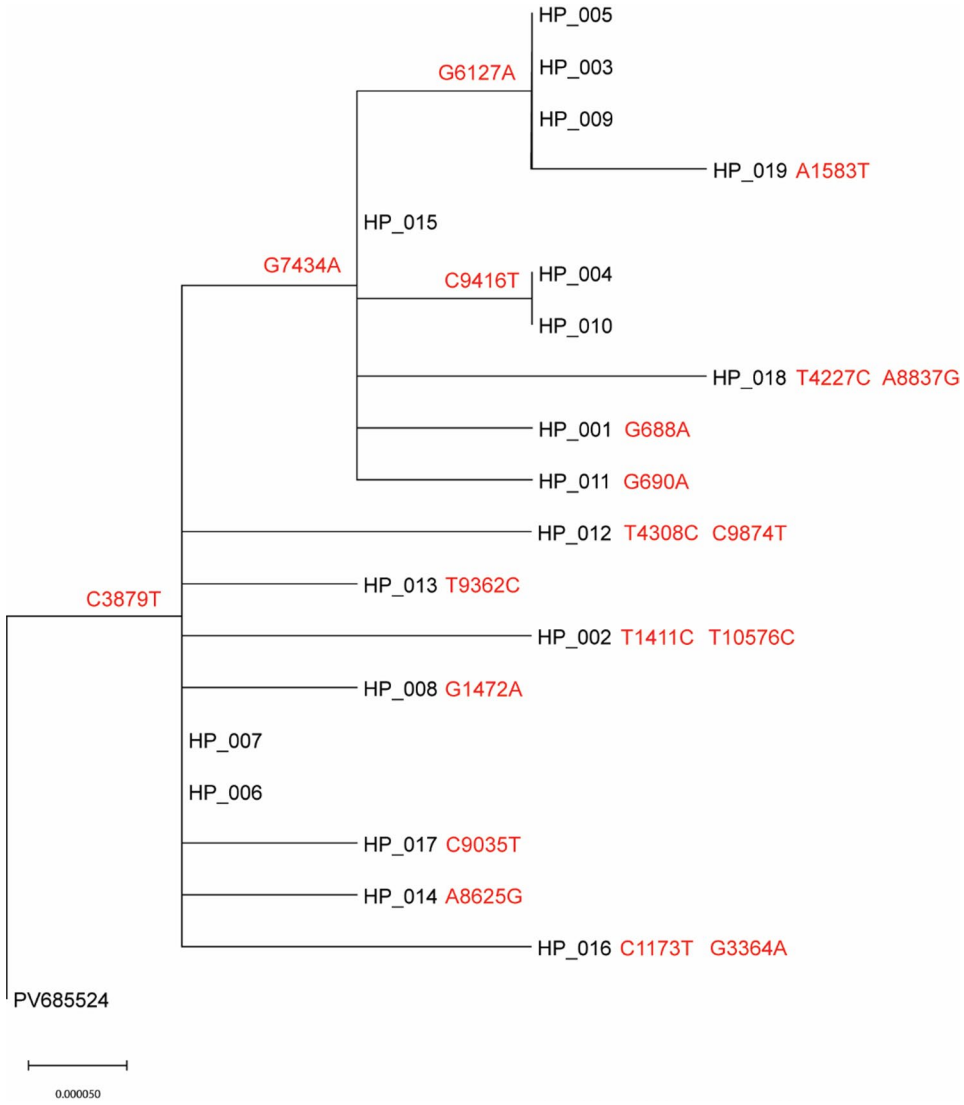

Fig. 2 Phylogeographic tree of 19 CHIKV genomes isolated in Guangzhou city. This tree was built by Maximum-Likelihood method. Nucleotide point mutations are highlighted in red; the mutations are relative to reference strain S27b03 (PV685524.1). Numbers indicate genomic positions

Table 3 Mutations and their effects on protein structure stability

Regions	Missense mutation	ΔΔGStability (kcal/mol)	Isolate_ID
nsP1	G230R	−0.72	HP_001
	R491Q	−0.35	HP_008
	Q528L	0.49	HP_019
nsP2	V587M	−0.25	HP_016
nsP4	A180T	−1.68	HP_019
			HP_009
E2	I54V	−1.13	HP_014
6 K	A47V	−0.88	HP_012
E1	V220A	−0.5	HP_002

In this work, several nonsynonymous substitutions were identified during local transmission, including G230R, R491Q and Q528L in nsP1; V587M in nsP2; A180T in nsP4; A47V in 6 K; I54V in E2; and V220A in E1. Previous research has reported that CHIKV harboring the nsP1 double mutation G230R and K299E presented markedly increased resistance to FHNA compared with the that of wild type [41]. Although isolate HP_001 contained the G230R mutation, no evidence of onward spread has yet been observed, highlighting the importance of continuous genomic detection. DynaMut2 was used to assess the relationship between amino acid substitutions and protein stability [38, 46]. Notably, five mutations including nsP1-G230R, nsP4-A180T, 6 K-A47V, E2-I54V, and E1-V220A showed $\Delta\Delta G \leq -0.5$ kcal/mol, indicating destabilizing effects. Such changes in structural stability could affect viral properties such as replication efficiency or transmissibility. For example, in SARS-CoV-2, the nucleocapsid mutation R203K/G204R was shown to increase viral replication and infectivity through protein destabilization [47]. Similarly, in

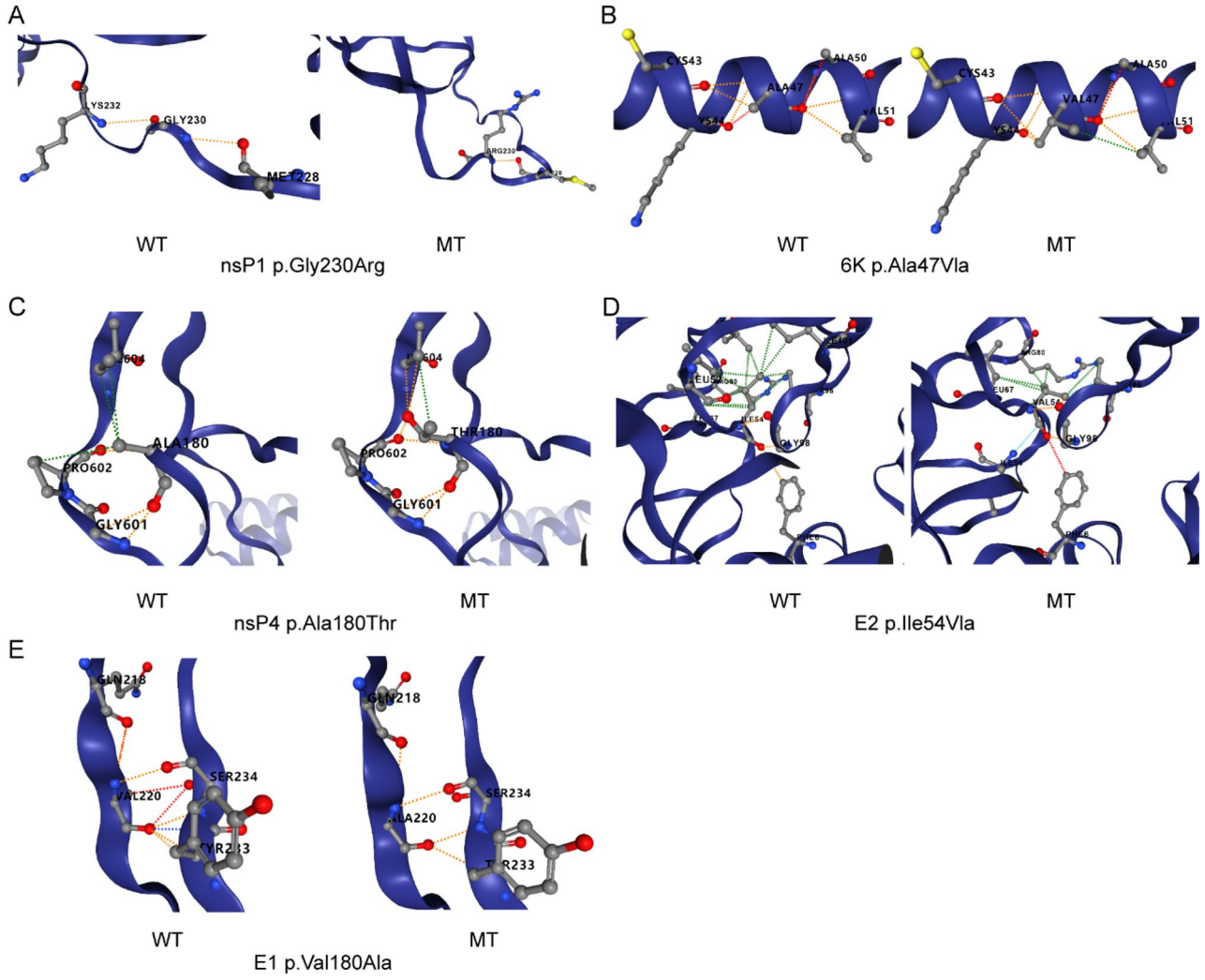

Fig. 3 DynaMut2 prediction of interatomic interactions of the native protein (WT) vs. the variant (MT). The Dots represent the inter-residue interaction with the following color codes: pink, clash; light blue, Van der Waals force; red, hydrogen bond; yellow, ionic; light green, aromatic; green, hydrophobic; blue, carbonyl contacts

Coxsackievirus B3, the VP1-F106L mutation reduces capsid stability and attenuates pathogenesis in mice, but increases replication kinetics in cell culture by facilitating genome uncoating [48]. In conclusion, our findings provide useful information for future functional studies on how genetic mutations influence CHIKV fitness and transmission dynamics.

This study has several limitations. First, the functional impact of the identified nonsynonymous mutations was inferred from computational structural predictions, and experimental studies will be required to confirm their effects on protein function or viral phenotype. Second, detailed epidemiological linkage information, such as travel history, symptom onset dates, and contact tracing records, was not available, which limited our ability to link genomic data to transmission dynamics. Finally, the sample size was relatively small and collected within a short timeframe, which may not fully capture the genetic diversity of circulating CHIKV strains in Guangzhou. Future studies with expanded sample coverage and integrated epidemiological data will provide a more comprehensive understanding of CHIKV transmission and evolution in Guangdong Province.

Conclusions

In conclusion, our work successfully demonstrated that CHIKV circulating in Guangzhou belongs to the ECSA lineage, and that the sequences are similar to those of the S27b03 strain. These isolates may share a common origin, as they all carry a c.3879 C > T substitution. Moreover, the identified genetic and amino acid mutations highlight features of local viral transmission and provide a basis for future investigations. These findings underscore the importance of continued genomic surveillance

in Guangzhou and surrounding regions. The presence of multiple nonsynonymous mutations, particularly in replication-associated proteins, highlights the need for monitoring potential changes in viral fitness or drug susceptibility. Ongoing surveillance combining epidemiological and genomic data will be essential for early detection of emerging variants.

Abbreviations

CHIKV	Chikungunya virus
ECSA	East/Central/South African
IOL	ECSA-Indian Ocean Lineage
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
FHNA	6-fluoro-homoneplanocin A
ONT	Oxford Nanopore Technologies

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-03051-8.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions

Conceptualization: Q.C. and Y.Z.; methodology: Y.Z., Z.Z., and Y.H.; software: Y.Z., Z.Z., and Y.H.; validation: Y.Z., Z.Z., X.Z., X.M., L.L. and Y.H.; formal analysis: Y.Z.; investigation: Q.C., Z.Z., X.Z., X.M., L.L. and Y.H.; resources: Q.C.; data curation: Y.Z.; writing-original draft preparation: Y.Z.; writing-review and editing: Y.Z. and Q.C.; visualization: Y.Z.; supervision: Y.Z. and Q.C.; project administration: Q.C.; funding acquisition: Q.C. All the authors have read and agreed to the published version of the manuscript.

Funding

Not applicable.

Data availability

The raw FASTQ files from nanopore sequencing in this study have been submitted to the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA1314437.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Guangzhou Huangpu District Center for Disease Control and Prevention (protocol code HPCDC20250010). The committee determined that the project did not involve harm to humans, sensitive personal information, or commercial interests, and thus ethical review was waived.

Consent for publication

All samples used in this study were anonymized and could not be traced back to individual donors. The donors were informed in advance that their samples would be tested for CHIKV and that the results could be used for research and publication purposes. Consent for this use was obtained before testing. No personal information was collected or included in this study.

Competing interests

The authors declare no competing interests.

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